June 30, 1949.

Dear Howard,

Your letter concerning "duplex" prototrophs followed directly on the heels of one from L. Cavalli (in Fisher's lab. at Cambridge) who raised exactly the same point, concerning essentially the same strains. However, W-677 does not carry Het.

My 1947 Genetics paper seems to suggest that duplex prototrophs occur rarely (1/90) if at all (Cf p. 520). In Dec. 1947, I went into the matter again, performing the crosses (e.g. Y-40 x W-1... this is a Mal- mutant of Y-53) on EMS Lac and EMS Mal with B_1. Here, the duplex colonies can be identified because they are sectored. Some of the data (exp. 51a) were:

	-	-	sectored.	% s.	% of - s.
Lac	99	189	5	1.7	5
Mal	37	529	7	1.2	19.

At that time, I thought that these data indicated that as many as 20% of the zygotes were yielding duplex prototrophs, but that these might often be difficult to detect when each crossover was in the same region (i.e., both segregants might be - or both +.) However, Mal has since shown an extraordinary behavior, which makes question any generalization based upon it. The published map distances would suggest, however, an appreciable frequency of prototrophs duplex for Lac if all of the segregants from a four-strand system were produced. In my hands, Lac duplex prototrophs, on dilute plates, have never been more than one or two percent of the total! I would be very much interested to have a full protocol of your procedure to compare it with present methods. Like yourself, I have worried how to prove that the duplex represent sister segregants. In a later experiment, on a larger scale, (exp. 64) 5 sectored, 17# and 512- colonies were counted. A total of 52 sectored and 130# were noted altogether (most of the plates were not counted for -). 45 of the Mal-sectored colonies were streaked out to separate the Mal- from Mal# components. There seemed to be a definite correlation as shown in this 2x2 table:

	M-L-	M-L#
M#L-	19	5
M#L#	4	17.

That is, where the M- was L-, the M# tended to be L-also, and similarly when M# was L#.

In addition, the Lac-V_1^r and Lac#V_1^s classes were markedly deficient among the duplex prototrophs as compared with the others. This certainly seems to bear the notion that the duplexes are the result of accidental juxtaposition of independent recombinants of a fairly high order. Mal is not linked to Lac (at least not in linear order). Except for the possibility of coincidence (which is very parallel here to Lindegren's old observations on N. crassa) another simple way to

way to test duplex significance would be to compare the rate of its occurrence on minimal and on thiamin agar. Owing to the close linkage (10 units) of B_1 to BM which necessitates a crossover to produce a full prototroph, omitting thiamin should diminish the proprotion of duplex 10-fold. I have noticed Mal-sectored prototrophs on minimal agar, and thought they were much less frequent than on thiamin, but haven't really done a proper experiment.

Frankly, I have become quite leery of the whole linkage system because of the peculiar behavior of the Het heterozygotes. Mal, as you've read, is almost invariably hemizygous, usually Mal-. Also, most prototrophs from the usual cross are Mal-(from comparable parents). However, Cavalli and I agree that Mal cannot be placed in linear linkage with the other factors. If it is on an independent chromosome, this chromosome does not segregate properly. I think that the defici of Mal# in prototrophs should be correlated with the loss of the segment carryin Mal# in the heterozygotes, but I haven't so far worked out a suitable mechanism to explain it. The rub is that I don't know to what extent the unequal segregati I find in the persistent heterozygotes may reflect what is going on always, so the whole question is up in the air, as far as the details of the linkage mechan are concerned. Mal isn't the whole story, as I've found one diploid heterozygous for Mal, in several hundred tests, and it also segregates abnormally for Mal and for other factors. Gal_1 (tentative symbol) is also usually hemizygous, but less uniformly Gal- in the heterozygotes, and not closely linked to Mal. So here is another region which may turn up deficient in the heterozygotes, and dis turn the normality of the segregation. But I hope to turn up some siploids event whose segregation will not be disturbed. Until the story is cleaned up, any linkage calculations will have to be treated with some reserve. That isnnot to say, however, that tests for allelism, and so forth, cannot be done quite straig forwardly, and I presume that this is what you have in mind for your streptomyci work. Any linkages that you pick up with Lac, V_1, (T-1) or B_1 probably will be ok also, and in fact, I would appreciate any lead that you might pick up on this which would be very useful for the laborious work on the heterozygotes.

I read your paper in J. Bact. on sr, etc., with special interest in the leaning that you show now towards a nuclear-segregation hypothesis. Judging from our correspondence of the last year or two, I would gather that you didn't accept this very strongly before because of the zero-points with B/1. I am still not convinced that at least some of the zero-points may be artefacts. To turn th argument the other way round, I was a little surprised that your paper did not bring in the possibility of some delay in the action of streptomycin. Do you have any information on the rate at which cells are killed? Could such an effect compensat for a delay in phenotypic expression? One of my students is working with Demere this summer, and when he returns, I expect will try to test the dominance of sr and sd in the heterozygotes, although I think that the present material is somewhat unsatisfactory because of the aneuploidy.

Be all this as it may, I am especially interested to learn of the unusually high frequency of duplex prototrophs that you've been running in to, and would appreciate as many details as possible so that I can try to reproduce it. We hop to go into it somewhat as an angle on our heterozygote studies, but will gladly (even eagerly!) leave it to your competent hands.

Yours sincerely,

Joshua Lederberg